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STUDIES TO CONTROL ENDEMIC TYPHOID FEVER IN CHILE

Annual Report

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A multi-faceted program of applied research has been undertaken in collaboration with the Ministry of Health of Chile intended to lead to control of endemic typhoid fever in Santiago, Chile. These studies include: 1) Maintenance of prospective epidemiologic and bacteriologic surveillance in three large-scale field trials evaluating the efficacy of Ty21a live oral typhoid vaccine given in various formulations and immunization schedules. 2) The first evaluations of Ty21a vaccine in infants and pre-school children. (continued)

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Figure 2

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3) Development of a new enzyme-linked immunosorbent assay (ELISA) to measure Vi antibodies and its use as a serologic screening test to identify chronic typhoid carriers. 4) Evaluation of a new oral antibiotic regimen to eradicate the chronic typhoid carrier state without resort to surgery.

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SUMMARY

A multi-faceted program of applied research has been undertaken in collaboration with the Ministry of Health of Chile intended to lead to control of endemic typhoid fever in Santiago, Chile. Information derived from these studies is directly applicable to the prevention of typhoid fever in United States military personnel deployed in endemic areas. During the past contract year activities that were emphasized include:

1) Maintenance of prospective epidemiologic and bacteriologic surveillance in three large-scale field trials evaluating the efficacy of Ty21a live oral typhoid vaccine given in various formulations and immunization schedules.

2) The first evaluations of Ty21a vaccine in infants and pre-school children.

3) Development of a new enzyme-linked immunosorbent assay (ELISA) to measure Vi antibodies and its use as a serologic screening test to identify chronic typhoid carriers.

4) Evaluation of a new oral antibiotic regimen to eradicate the chronic typhoid carrier state without resort to surgery.

Field Trials with Ty21a

Results of the large-scale field trials of Ty21a show that an enteric-coated capsule formulation is significantly superior in efficacy to a gelatin capsule/$NaHCO_3$ formulation. One dose of vaccine in enteric-coated capsules provides only low levels of short-lived protection while the moderate (65%) protection conferred by two doses lasts for only two years. Three doses of Ty21a in enteric-coated capsules given within one week provides moderate protection (65%) for at least three years and therefore is equal in efficacy to the level of protection provided by heat-phenol-inactivated parenteral whole cell vaccine; the live oral

vaccine, however, has the distinct advantage of not causing adverse reactions. Four doses of Ty21a vaccine in enteric-coated capsules provides significantly superior protection than three doses.

Ty21a Vaccine in Infants and Pre-School Children

Preliminary studies in a total of 127 children less than five years of age who received three or four doses of Ty21a vaccine or placebo in milk with bicarbonate show that the vaccine is very well-tolerated and causes no identifiable adverse reactions. However, in these very young children the vaccine proved to be poorly immunogenic, using serologic assays that show high seroconversion rates in older children and young adults in Chile.

A New Serologic Screening Test for Chronic Typhoid Carriers

Tyraminated Vi polysaccharide was used as antigen in an ELISA to detect Vi antibody. IgG class Vi antibodies were present in a titer of 1:200 in 0 of 22 healthy U.S. adults, 2 of 17 (12%) Chilean adults with acute typhoid fever, but in 44 of 51 (86%) chronic typhoid carriers. Sera of 141 Santiago foodhandlers were tested for Vi antibody at a dilution of 1:200 and each individual had two stool cultures to detect S. typhi. The only individual with a positive titer for IgG antibody proved to be a chronic carrier with positive stool cultures; the other 140 women had negative stool cultures.

A New Therapy for the Chronic Typhoid Carrier State

Oral ciprofloxacin at a dose of 750 mg twice daily for 28 days was evaluated in 12 patients as a therapy to eradicate the chronic carrier state. One patient's cultures failed to clear after therapy, while another had a re-infection after six months of negative cultures with an new organism having a distinict phagetype. In the remaining 10 patients, stool and bile cultures have remained negative for at least six months.

TABLE OF CONTENTS

TABLE OF CONTENTS
(continued)

I. INTRODUCTION

Typhoid fever remains an important public health problem in many less-developed regions of the world and poses a risk for travelers from industrialized countries who visit such endemic regions. In virtually all endemic areas the incidence rates for typhoid fever are highest in children 5-19 years of age, i.e. schoolchildren. This is of potential relevance in the control of typhoid, since schoolchildren represent a "captive" population amenable to school-based immunization programs.

For United States military personnel who are stationed in less-developed areas or who must be prepared at short notice to operate in less-developed areas of geopolitical importance, typhoid fever represents an important potential health risk. The current vaccine utilized by the U.S. military forces to prevent typhoid fever, an acetone-inactivated preparation of whole Salmonella typhi inoculated parenterally, requires at least two doses given several weeks apart to immunize and causes high rates of significant adverse reactions. Therefore, a high priority has been given to identifying alternative typhoid vaccines that will provide significant protection without causing notable adverse reactions.

In areas where typhoid fever is endemic, the prevalence of chronic gall bladder carriers of S. typhi is often quite high. Thus, a particularly onerous risk of transmission of typhoid fever to U.S. military personnel in less-developed areas comes from foodhandlers from the indigenous population who may be chronic typhoid carriers and who unknowingly are involved in preparation of food. Under these circumstances, unwittingly, the potential exists for large epidemics to

occur. Furthermore, the size of the inocula of S. typhi present in food vehicles may be sufficiently high to overcome the protective efficacy of the current acetone-inactivated parenteral vaccine. Consequently, a simple, practical yet sensitive and specific screening test is required to screen large groups of individuals for the presence of suspected chronic typhoid carriers.

Dependents, including children, who accompany U.S. military personnel stationed on tours of duty in less-developed countries must also be protected against typhoid fever. In young children the subject of adverse reactions to the current parenteral typhoid vaccines is even more pertinent.

For the past several years, the Center for Vaccine Development of the University of Maryland has conducted an applied research program on the control of typhoid fever in Santiago, Chile, a highly endemic area. During the past two years, the program has,in particular, concentrated on field studies with Ty21a live oral typhoid vaccine, the development of improved serologic screening tests for the chronic typhoid carrier state, the identification of improved non-surgical methods to treat chronic carriers, and evaluations of Ty21a vaccine in infants and young children (representing the first experiences with this vaccine in children less than six years of age). Results of these studies have direct relevance for the improved prevention of typhoid fever in U.S. military personnel.

II. FIELD TRIALS OF EFFICACY OF LIVE ORAL TYPHOID VACCINE TY21a

A. Field Trials with Parenteral Killed Whole Cell Typhoid Vaccines

Parenteral killed whole cell typhoid vaccines have been available since 1896 and have been used throughout this century. In the 1950s and 1960s the World Health Organization sponsored a series of large-scale field trials in several countries to assess the efficacy of various types

of parenteral killed whole cell vaccines. In the first of these trials, in Yugoslavia, a fluid heat-inactivated, phenol-preserved parenteral vaccine was found to be superior in protective efficacy in comparison with an alcohol-inactivated and preserved vaccine (1,2).

Shortly after results of the above field trials in Yugoslavia became available, the Walter Reed Army Institute of Research in Washington, D.C. prepared for the World Health Organization two lyophilized reference vaccines for use in several additional field trials (3). These included a heat-phenol-inactivated and an acetone-inactivated vaccine, referred to resepectively as L and K vaccines. The reference L and K vaccines were evaluated together in randomized, controlled, double blind trials in Yugoslavia and Guyana(4,5); in addition, the K vaccine was tested for efficacy in controlled trials in Poland and the L vaccine in the U.S.S.R. (6,7). Results of these trials are summarized in Table 1. While both reference vaccines conferred significant protection in the field trials in Yugoslavia and Guyana, the K vaccine was found to provide significantly superior protection than the L vaccine. In three separate trials, L vaccine conferred 51% (Yugoslavia), 66% (U.S.S.R.), and 67% (Guyana) protection (Table 1).

Although the acetone-inactivated vaccine showed somewhat greater protection than the heat-phenol-inactivated vaccine, acetone-inactivated vaccine is largely unavailable. Of the manufacturers of parenteral killed whole cell typhoid vaccines listed in the WHO's International List of Availability of Vaccines and Sera (8), 40 make the heat-phenol-inactivated variety while only three manufacture the lyophilized acetone-inactivated vaccine. Thus, for most of the world the heat-phenol-inactivated vaccineis what is available as an option for use in public health

programs. In fact, however, parenteral killed whole cell typhoid vaccines are rarely used by any country in systematic typhoid fever control programs because of the high rates of adverse reaction that they elicit. A summary of the adverse reaction rates encountered in the WHO-sponsored field trials of K and L vaccines in Yugoslavia (4), Guyana (5), and the U.S.S.R. (7) are shown in Table 2.

B. Development of Ty21a Live Attenuated Oral Vaccine

An important advance for the potential control of typhoid fever was the development by Germanier and Furer of an attenuated strain of S. typhi, Ty21a, that can be utilized as a live oral vaccine (9). In preliminary studies in adult volunteers in North America, Ty21a was found to cause no adverse reactions (in contrast to the parenteral killed whole cell vaccines), to be genetically stable, and to significantly protect against experimental infection with an inoculum of pathogenic S. typhi that caused typhoid fever in 53% of control volunteers (10).

Based on these highly encouraging observations in adult volunteers, Ty21a vaccine was evaluated for efficacy by Wahdan et al (11,12) in a placebo-controlled, randomized, double-blind trial in Alexandria, Egypt. In this trial, three doses of Ty21a vaccine ($1-3 \times 10^9$ viable vaccine organisms per dose) or placebo were given to the children on Monday, Wednesday, and Friday of one week. Prior to ingestion of vaccine or placebo, children chewed a tablet containing 1.0 gm of $NaHCO_3$ (to neutralize gastric acid). Each dose of lyophilized vaccine or placebo was contained within glass vials in vacuo. The vials were opened, the lyophilate reconstituted in the field with diluent, and the liquid vaccine (or placebo) suspension given to the child a few minutes after the child ingested the $NaHCO_3$ tablet. Passive surveillance failed to identify

notable adverse reactions, corroborating the safety of the live oral vaccine.

During the 36 month period of surveillance in Alexandria, the efficacy of the vaccine was 96% (Table 3) (11).

Shortly after the Egyptian field trial established the biological safety and efficacy of Ty21a in schoolage children in an endemic area, the Swiss Serum and Vaccine Institute made a formulation of vaccine commercially available which consisted of two gelatin capsules each containing 0.4 gm of $NaHCO_3$ and a third gelatin capsule containing lyophilized vaccine. Although this formulation resembled that used in Alexandria, Egypt, it was clearly not identical. Despite the highly encouraging results in the first field trial in Egypt, it was obvious that additional information had to be obtained before the Ty21a live oral vaccine could be employed as a practical public health tool. Some of the critical further questions to be answered included:

1) What was the efficacy of Ty21a when administered in a formulation such as enteric-coated capsules that does not require pretreatment with $NaHCO_3$?

2) Could fewer doses (one or two) than used in Alexandria provide a satisfactory level of protection?

3) What level of protection would Ty21a provide in areas with incidence rates of typhoid fever much higher than the 44-50 cases/10^5 that prevailed during the trial in Alexandria?

4) What is the efficacy of the commercial formulation consisting of gelatin capsules containing $NaHCO_3$ and lyophilized vaccine that was marketed after the Egyptian field trial?

5) Could prolongation of the interval between the doses enhance

the immunogenicity of the vaccine?

6) Could an immunologic assay be identified that would correlate with levels of vaccine efficacy in field trials and could therefore be used to predict the effect of changes in formulation and immunization schedules?

In order to answer these questions, three separate field trials of efficacy have been carried out in Santiago, Chile (with a fourth scheduled to begin in October, 1986). These trials represent a collaborative effort undertaken by the Ministry of Health, Santiago, Chile, the Center for Vaccine Development of the University of Maryland School of Medicine, the Pan American Health Organization, the World Health Organization, the Swiss Serum and Vaccine Institute, and the Walter Reed Army Institute of Research.

C. Materials and Methods of the Field Trials in Santiago, Chile of Ty21a Vaccine

The first field trials were placebo-controlled and were initiated in the Northern (Area Norte) and Western (Area Occidente) administrative areas of Santiago in 1982 and 1983, respectively. The third field trial was begun in the Southern (Area Sur) and Central (Area Central) administrative areas of Santiago in 1984. Santiago, Chile was selected as the site for these field trials because of the combination of high endemicity of typhoid fever (the annual incidence rate from 1977 to 1981 exceeded 150 cases per 10^5 population), the presence of an internationally-reknowned health care infrastructure (the National health Service), a strong commitment on the part of the Ministry of Health towards innovative methods to control typhoid fever, and a long history of school-based vaccination programs.

Only children of consenting parents entered the studies and were

randomized to the various cells of the trials. Remaining children of non-consenting parents were also kept under surveillance and served as unvaccinated controls. Randomization occurrred by classroom.

Since typhoid fever exhibits a marked seasonality (November to April) in conjunction with summer in Santiago, the vaccinations were limited to the cool months of the year (May to October). Computerized data files were generated from the completed class lists.

In the Chilean field trials only bacteriologically-confirmed cases (i.e. those from whom S. typhi was isolated from blood, bone marrow, or bile-stained duodenal fluid) were utilized in computations of vaccine efficacy. Therefore considerable resources were directed toward bacteriologic confirmation of suspect cases. Children admitted to hospital with a clinical suspicion of typhoid fever had three 4 ml blood cultures and one bone marrow culture obtained (13). Children presenting to the consultorios (health centers) as outpatients with suspect typhoid fever had two blood cultures drawn 30 minutes apart. S. typhi and S. paratyphi A and B isolates were sent for phage typing to the Institute of Public Health in Santiago and to the WHO Collaborating Center for Phage Typing of Salmonella, Division of Enteric Pathogens, Central Public Health Laboratory, Colindale, London, England.

D. Results of the Field Trials of Ty21a Vaccine in Santiago, Chile

Chronologically, the Area Norte field trial preceeded the Area Occidente field trial. However, for purposes of clarity of presentation, the sequence of presentation of data will be Area Occidente, followed by Area Norte, and finally Area Sur and Central.

1. Area Occidente Field Trial

Parents of 141,127 children (representing 96% of all schoolchildren in

Area Occidente) consented for their children to participate and these were thereupon randomized to one of five groups to receive:

Group 1 - Three doses of vaccine in enteric-coated capsules given with an interval of two days between the doses.

Group 2 - Three doses of vaccine with $NaHCO_3$ given with an interval of two days between the doses. The commercial gelatin capsule formulation was used which consisted of two gelatin capsules each containing 0.5 gm of $NaHCO_3$ and a third gelatin capsule containing lyophilized vaccine.

Group 3 - Three doses of vaccine in enteric-coated capsules with an interval of 21 days between the doses.

Group 4 - Three doses of the commercial gelatin capsule formulation with an interval of 21 days between the doses.

Group 5 - Three doses of placebo given with an interval of two days between the doses.

Mass administration of vaccine (containing $1-3 \times 10^9$ viable vaccine organisms per dose) or placebo was carried out between mid July and mid September, 1983 and surveillance began on September 21, 1983. In total, 115,481 children received all three scheduled doses of vaccine or placebo.

Results of three years of surveillance in the Area Occidente field trial are shown in Tables 4 and 5. The main points are:

1) The enteric-coated formulation was very significantly superior to the gelatin capsule/$NaHCO_3$ formulation (Table 4).

2) Increasing the interval between doses to 21 days offered no advantage to administering all three doses within one week (Table 4).

3) Three doses of enteric-coated vaccine administered within one week provided similar levels of protection during three consecutive typhoid seasons. Thus protection with this regimen is protective for at least

three years (Table 5).

4) The level of protection conferred by the best regimen in the Occidente field trial (three doses of enteric-coated capsules given within one week) was 67% during three years of surveillance. This is less than the 96% protection for three years provided by three doses of a liquid formulation in the Alexandria, Egypt trial. However, the mean annual incidence rate in the placebo control group in the Occidente trial ($103/10^5$/year) was twice as high as the mean annual incidence rate recorded in the placebo group in the Alexandria trial ($46/10^5$). These data demonstrate that the enteric-coated capsule formulation of Ty21a protects even in areas where the incidence of typhoid fever is high.

Surveillance is being maintained in Area Occidente to determine if the efficacy of Ty21a can endure for more than three years. This information is critical for public health authorities to be able to design typhoid control programs based on the systematic use of Ty21a.

2. Area Norte

Parents of 92,356 of the 137,697 schoolchildren in Area Norte gave permission for their children to participate and these children were randomized to one of three groups to receive:

1) Two doses of Ty21a vaccine in enteric-coated capsules ($1\text{-}3 \times 10^9$ organisms per dose).

2) One dose of vaccine and one dose of identical appearing placebo.

3) Two doses of placebo.

The two doses of vaccine or placebo were given to the children one week apart in May and June, 1982 and surveillance began on July 1, 1982.

Results of the Area Norte field trial are shown in Table 6. The main points include:

1) Two doses of enteric-coated vaccine provided moderate (48-72%) protection for a period of two years. However, the efficacy then dropped to 21% in the third season and was non-existant by the fourth season of surveillance.

2) A single dose of vaccine in enteric-coated capsules provided low levels of protection (16-39%) for two years but by the third year of surveillance no further efficacy was demonstrable.

These data demonstrate that, when administered in enteric-coated capsules, Ty21a provides insufficient levels of protection when given as only one or two doses.

3. Area Sur and Area Central Field Trials

A third field trial was undertaken in 1984 in Areas Sur and Central where 248,544 children were randomized to receive either two, three or four doses of Ty21a vaccine (1-3 x 10^9 viable vaccine organisms per dose) in enteric-coated capsules with all doses of vaccine being administered within a period of eight days in September and October, 1984. No placebo control group was included in this trial in which surveillance began on November 1, 1984.

Results of surveillance of typhoid fever through two seasons are shown in Table 7. In this trial the incidence of typhoid fever in recipients of three doses of Ty21a in enteric-coated capsules was only slightly lower than the incidence in children who received two doses of vaccine. In contrast, the incidence of typhoid fever in recipients of four doses of vaccine was very significantly lower than the rates in children who received two or three doses.

4. Area Sur Oriente Trial

Preparations are underway to carry out a fourth field trial of

efficacy of Ty21a in the Sur Oriente administrative area. In this trial children will receive three doses of Ty21a in either enteric-coated capsules or in a liquid formulation or they will receive placebo in liquid or enteric-coated formulations. Results of this trial should answer the question of whether a liquid formulation of Ty21a similar to what was used in Egypt is inherently superior to Ty21a in enteric-coated capsules. By comparison of rates in the vaccine groups with the rate in the placebo group, this trial will also provide information on the absolute efficacy conferred by each formulation of vaccine.

E. Secular Trends of Typhoid Fever in Santiago and Evidence for an Epidemiologic "Herd Immunity" Effect Consequent to the Broad Application of Ty21a Vaccine

Analysis of the incidence rate of typhoid fever in the placebo control group in the first field trial of Ty21a in Area Norte, Santiago provides some fascinating insights on what might be expected from the systematic wide-scale application of Ty21a live oral vaccine in typhoid fever control programs. As seen in Table 6, the incidence rate in the randomized control group in the first year of surveillance was 210 cases/10^5 schoolchildren. This rate of culture-confirmed cases is similar to the reported rate for schoolchildren in Area Norte in the period 1977-1981, prior to the field trial; however, at that time cases were not bacteriologically confirmed.

Surveillance of the second typhoid season in Area Norte took place after more than 115,000 children in the adjacent area, Area Occidente, were entered into a field trial involving three doses of vaccine given in two different formulations and in two distinct immunization schedules. The incidence rate in the placebo control group in Area Norte in this

second year of surveillance fell to 141 cases/10^5 (Table 6).

Shortly before the third typhoid season of surveillance began in Area Norte, more than 248,000 children in Areas Sur and Central were given two, three or four doses of vaccine. In this third year of surveillance the incidence in the placebo group in Area Norte was seen to fall even further to 69 cases/10^5 (Table 6). A rate this low had not been encountered in Area Norte for decades.

The fourth year of surveillance in the Area Norte field area occurred during a year when no further trials were carried out in Santiago. Notably, in that year in which there were no large-scale administrations of vaccine, the incidence of typhoid fever in the placebo control group did not fall further. Rather, the incidence, 78 cases/10^5, closely resembled that of the previous year (Table 6).

In the course of the three field trials carried out so far in Santiago, approximately 65% of the schoolchildren in the city have been entered into field trials, many having received an efficacious formulation and number of doses of vaccine. One possible interpretation of the secular trends of incidence of typhoid fever in Santiago from 1982 to 1986, as shown by the incidence rates in the placebo control group in the Area Norte trial, is that the falling incidence is due to mass application of Ty21a vaccine in schoolchildren. As seen in Table 6, when Ty21a was given in field trials in other areas the incidence in the control group in Area Norte fell. In the fourth year of surveillance in Area Norte, when no further vaccine trials were carried out in Santiago, the incidence rate stabilized. Based on these observations, we would predict that the incidence of typhoid fever in the control group in Area Norte will diminish further in the fifth year of surveillance as children are

vaccinated in the field trial in Area Sur Oriente.

F. Correlation of IgA ELISA S. typhi O Antibody with Efficacy in Field Trials

During the past several years we have been carried out serologic studies in healthy young adult Chileans, age 17-21, who have been recipients of Ty21a in one of two formulations and in various immunization schedules. Serum IgG and IgA antibodies to S. typhi O antigen have been measured by ELISA before and after vaccination. Now that results of the field trials are available, it has become possible to relate seroconversion rates to vaccine efficacy; these comparisons are summarized in Table 8. It is obvious that there exists an excellent positive correlation between seroconversion rate of IgG S. typhi O antibody and vaccine efficacy in the field.

At present we conclude from all available and relevant data that the operative mechanism of protection conferred by Ty21a is mediated by cell-mediated immune mechanisms directed against the O antigen. While IgG O antibodies may not be protective per se, the high rates of seroconversion of these antibodies in populations orally vaccinated with Ty21a clearly correlate with stimulation of protective immunity.

G. Discussion

The great advantage of Ty21a live oral typhoid vaccine, in comparison with parenteral killed whole cell vaccines, is that it provides significant protection without causing adverse reactions. A wealth of evidence from volunteer studies and from some of the largest vaccine field trials ever carried out attest to the biological activity of this attenuated strain in providing protection against typhoid fever. Considerable resources have been expended in attempts to identify an

effective and practical formulation and dosage schedule for Ty21a. After a series of field trials in Egypt and Chile, considerable information has now been accrued demonstrating both the advantages as well as the limitations of Ty21a.

In Alexandria, Egypt, three doses of a liquid formulation of vaccine provided 96% protection for three years in an area where the incidence of typhoid in the control group was 46 cases/10^5(11). Field trials inChile have shown that Ty21a in enteric-coated capsules is significantly more protective than vaccine administered in the gelatin capsule/$NaHCO_3$ formulation. In the Chile trials, three doses of an enteric-coated formulation of Ty21a given within one week have provided 67% protection for at least three years. Giving fewer (i.e. one or two) doses of vaccine in enteric-coated capsules conferred inferior levels of protection that were short-lived, while administration of four doses of vaccine in enteric-coated capsule provided significantly greater protection than three doses. A fourth field trial currently beginning in Chile, as well as a field trial of similar design in Indonesia, will answer the question of the relative efficacy of enteric coated capsules versus a liquid formulation.

The 67% protection conferred by three doses of enteric-coated capsules given within one week in Chile is notably less than the impressive 96% efficacy over three years of a liquid formulation in Alexandria, Egypt. Nevertheless, in our estimation, this level of protection clearly makes Ty21a the vaccine of choice at present for any country intending to embark on a systematic typhoid fever control program. We conclude this because the level of efficacy is similar to what can be achieved with the liquid heat-phenol-inactivated parenteral vaccine, the other widely available

effective vaccine. However, Ty21a is distinctly more advantageous because in enteric-coated capsules it is easy to administer and amenable to mass vaccinations of schoolchildren; furthermore, Ty21a causes no discernable adverse reactions (14). In contrast, the heat-phenol-inactivated vaccine causes notable adverse reactions in approximately 25% of recipients and must be administered by needle and syringe or jet gun; the rate of local adverse reactions increases with jet gun administration (15).

Analyzing the incidence rate of typhoid fever in the randomizedplacebo control group in the first (Area Norte) field trial shows strong evidence that widespread vaccination with Ty21a creates a "herd immunity" effect in which the incidence increasingly drops in the control group as children in other areas of the city are vaccinated. These observations support the contention that Ty21a live oral vaccine, while not the ideal anti-typhoid vaccine, is neverthless a credible weapon to be employed in systematic typhoid fever control programs. Since man is the only reservoir, as well as the only natural host, of this infection, this approach is epidemiologically rational.

The multiple field trials of efficacy of Ty21a that have been required so far to generate the information necessary to determine how to use this vaccine as a public health tool are reminiscent of the series of field trials undertaken by WHO over a period of more than 15 years to accrue similar information for the parenteral killed whole cell vaccines. Until a superior formulation of Ty21a is identified, or Ty21a is surpassed by another attenuated strain, the information now available should allow public health authorities to begin to consider Ty21a in enteric-coated capsule formulation as a tool to be employed in national typhoid fever

control programs.

III. STUDIES WITH TY21a ORAL VACCINE IN INFANTS AND TODDLERS

A. Background

Live oral typhoid vaccine Ty21a has proven to be an important advance for the prevention and possible control of typhoid fever in endemic areas because it provides significant protection without causing adverse reactions. Although typhoid fever in endemic areas is largely a disease of schoolage children, the main delivery system for pediatric vaccines in most developing countries is through the expanded program on immunization (EPI) which is heretofore usually targeted exclusively at infants. Thus it is intriguing to consider whether immunization of infants might protect these children later when they reach schoolage. To even consider such a proposition it will be necessary to show that Ty21a is immunogenic in infants and young children. Because of the innocuity of Ty21a and the propensity of Salmonella to avidly interact with M cells of gut lymphoid tissue, many investigators have introduced genes encoding putative protective antigens of other organisms to obtain expression in Ty21a, thereby using the attenuated S. typhi as a "carrier" bacteria. Among the combinations reported so far are Ty21a expressing Shigella sonnei O antigen (17), B subunit of Escherichia coli heat-labile enterotoxin (LT) (18), and an outer membrane protein of Vibrio cholerae (19). Important target age groups for these bivalent vaccines are also infants and young children. Heretofore, however, the youngest age group to have received Ty21a vaccine is six year olds. We therefore initiated studies to evaluate the clinical acceptability and immunogenicity of Ty21a in infants and young children (less than five years of age) in Santiago, Chile, an

area endemic for typhoid fever.

B. Materials and Methods

Vaccine was administered in three separate, randomized, placebo-controlled, double-blind studies.

1. Study #1

Study #1 involved healthy children 6-24 months of age recruited from the well baby clinic of the Centro Diagnostico of the Universidad Catolica School of Medicine, Santiago. Following explanation of the study to the parents and obtaining written consent, infants were randomized to receive three doses of vaccine (10^9 organisms per dose) or placebo which were given within eight days. Cups containing vaccine or placebo were prepared in a separate room by an unblinded nurse. She dissolved the contents of an enteric-coated capsule of Ty21a vaccine into 90 ml of cow's milk formula containing 0.5 gm of $NaHCO_3$. (A similar milk/bicarbonate "cocktail" method had been previously successfully used to vaccinate Chilean six year olds who demonstrated a good serologic response post-vaccination) (20). Milk containing $NaHCO_3$ alone served as the placebo. The coded cups containing vaccine or placebo were presented to a second nurse who administered the contents to the children in double blind fashion. The infants were examined 24 and 48 hrs after each dose of vaccine at which time the child's temperature was recorded; axillary temperatures were obtained because this is the accepted custom in Chile. The mother was interviewed to elicit evidence of adverse reactions in the previous 24 hrs.

A 4 ml specimen of blood was collected prior to and 21 days after vaccination. The blood was passed through a Ficoll-Hypaque column to obtain mononuclear cells to carry out lymphocyte replication studies with selected S. typhi and appropriate control antigens to measure the

cell-mediated immune response to vaccination with Ty21a. Plasma was utilized to measure serum antibodies: IgG antibody to O antigen was measured by enzyme-linked immunosorbent assay (20); H antibody was measured by Widal tube agglutination as previously described (21) and Vi antibody was detected by passive hemagglutination using highly purified Vi polysaccharide (22).

2. Study #2

This study was carried out among children 2-5 years of age (three-fourths were three or four year olds) in a nursery school in the Pincoya district of Area Norte, Santiago. Children of consenting parents were randomized to receive three doses of Ty21a vaccine (10^9 viable organisms per dose) or placebo given within a period of eight days. Capsules of vaccine were opened by an unblinded individual in a separate room and the contents suspended in 50 ml of cow's milk containing 0.75 gm of $NaHCO_3$; placebo consisted of milk with bicarbonate only. The coded cups containing vaccine or placebo were presented to a nurse who distributed them to the children in double blind fashion. Children were examined 24 and 48 hrs after vaccination at which time axillary temperatures were taken and the parents were interviewed.

Before vaccination and 21 days thereafter 4 ml of blood were collected and the sera separated and frozen to be tested later for antibody as described above.

3. Study # 3

The third study was carried out in 2-5 year old children in a second nursery school in Pincoya where children of consenting parents were randomized to receive four doses of Ty21a vaccine (10^9 viable organisms per dose) or placebo. Vaccine was administered identically as in Study # 2 but a fourth dose was given within the eight day period in attempt to

increase vaccine immunogenicity. Blood was collected before and 21 days after vaccination for serologic tests as described above.

C. Results

1. Clinical Response to Vaccine

Table 9 shows the number of children in each study who received vaccine or placebo and the frequency of adverse reactions. Diarrhea, fever, vomiting and abdominal pain were uncommon in either group with no difference evident between vaccine and placebo recipients.

2. Immune Response to Ty21a

The serologic response to vaccination of infants and young children is summarized in Table 10. In Study #1, involving infants and toddlers less than two years of age, no significant rises in O antibody measured by IgG-ELISA were detected. Because these results contrast so notably from the serologic response of six year olds administered Ty21a vaccine by this method of administration in a previous study (20), we proceeded in the next study to vaccinate slightly older children, 2-5 years of age. These children in Study # 2 showed some serologic reactions to both O and H antigens; in total 8 of 24 vaccinees showed a significant rise in one or another serologic test versus 0 of 25 pre-school children who received placebo ($p<0.002$).

In the third study, we attempted to increase the immunogenicity of the vaccine by administering an additional dose to preschool children. The addition of a fourth dose did not increase the serologic response to the vaccine.

Ty21a vaccine does not contain Vi antigen and therefore even in older individuals does not stimulate Vi antibodies. Thus the total lack of serologic response to Vi where measured in these studies in young children

is completely as expected (Table 10).

Lymphocyte cultures from the vaccinated and placebo infants responded to mitogens. However, the lymphocytes collected post-vaccination failed to show evidence of replication in the presence of S. typhi O polysaccharide or control (S. thompson or E. coli) O polysaccharides.

IV. A NEW SEROLOGIC TEST TO SCREEN FOR CHRONIC TYPHOID CARRIERS

A. Background

In the course of acute S. typhi infection, typhoid bacilli reach the gall bladder, an organ for which they have a remarkable predilection. In fact recovery of S. typhi from bile-stained duodenal string cultures has become an increasingly popular method of confirming the diagnosis of acute typhoid fever (23). In approximately 2-7% of individuals with acute S. typhi infection, depending on age and sex, a chronic gall bladder infection ensues and these individuals become carriers for life (24,25). The diagnosis of the chronic typhoid carrier state is made by recovering S.typhi from stool or bile cultures. Coprocultures must be done repeatedly because chronic carriers are notorious for having intermittent negative cultures.

Bacteriologic methods are necessary to confirm the diagnosis of the chronic carrier state but are totally impractical for screening large numbers of individuals. As a consequence screening tests to identify chronic carriers have been sought. It has been known for many years that chronic carriers of S. typhi have inordinately high titers of Vi antibodies. However, until recently, the lack of highly purified Vi antigen made most tests for Vi antibodies subject to unacceptable variability. Nolan et al (26) developed a passive hemagglutination assay (PHA) for Vi antibody which we (23) subsequently showed could be used successfully to screen for chronic typhoid carriers in an endemic area

(Santiago). The PHA, however, requires that test sera be initially absorbed with untreated sheep erythrocytes to remove any anti-sheep antibodies, prior to reacting the sera with sheep erythrocytes coated with Vi antigen. This is a cumbersome step that limits the number of specimens that can be examined. Because of this limitation, an alternative test, such as an ELISA, was sought that would be more practical and amenable to screening large numbers of sera. Heretofore, ELISA to measure Vi antibody has been hampered by the physicochemical properties of Vi polysaccharide causing it to bind poorly to the plastic of microtiter plates. Dr. John Robbins of the National Institute of Child Health and Human Development has recently solved this problem by preparing a tyraminated derivative of Vi that readily binds to microtiter plates.

B. Methods

Imulon 96 well microtiter plates were coated overnight with 0.1 ml of solution containing 1 mcg/ml of tyraminated Vi antigen in 0.015 carbonatebuffer pH 9.6.

The plates were washed with PBS-Tween and incubated at 37°C for 1 h with test serum containing 1% non-immune goat serum and 1% fetal bovine serum. The plates were washed with PBS-Tween and incubated with alkaline phosphatase conjugated goat anti-human IgG, IgA, or IgM. After washing, the ELISA was completed and color generated with p-nitrophenyl phosphate (1mg/ml) in 10% diethanolamine.

In order to standardize the assay and assess its sensitivity and specificity, sera from three separate populations were used including; 22 healthy young adult North Americans with no history of typhoid fever or vaccination; 17 teenage or young adult patients with bacteriologically-confirmed acute typhoid fever from the Infectious Diseases Hospital in Santiago; 51 bacteriologically-confirmed chronic

typhoid carriers from Chile; 141 healthy foodhandlers who prepare food in schools in Santiago.

C. Results

Table 11 shows the results of testing the various populations for IgG class antibody to Vi. At a serum dilution of 1:200, none of the 22 U.S. adults had positive titers, while only 2 of 17 (12%) patients with acute typhoid fever had titers. In contrast, 44 of the 56 (86%) chronic typhoid carriers were positive at this dilution and most, in fact, had much higher end point titers. The 141 healthy foodhandlers in Santiago schools were examined bacteriologically with stool cultures on two days as well as with serological tests. One individual was positive by IgG-ELISA at a 1:200 dilution of serum, while this individual and one other had PHA titers $\geq$ 160. Only the individual who was positive by ELISA was bacteriologically confirmed to be a typhoid carrier.

Results with the IgM and IgA ELISAs for Vi antibody are shown in Table and 13. It is obvious from the comparison with Table 12 that most serum Vi antibody in chronic carriers is in the IgG class.

V. A NON-SURGICAL DOMICILIARY ORAL REGIMEN FOR TREATING THE CHRONIC TYPHOID CARRIER STATE

A. Background

Chronic gall bladder carriers of S. typhi can be successfully treated with cholecystectomy combined with a course of antibiotics before and several weeks after surgery. Neverthless, this form of "therapy" is hardly an economical or practical public health tool and is not appicable to persons who are poor surgical risks. Many investigators have attempted to eradicate the chronic carrier state by various oral antibiotic regimens. However, the experience has shown that carriers with gall stones are extremely difficult to successfully treat. A trial previously

carried out as part of this research contract involved the administration of oral amoxicillin (2.0 gm) and probenecid (0.5 gm) three times daily for 28 days to 26 carriers, all of whom had gall bladder disease and most of whom had gall stones (27). In this study, 15 0f 26 carriers were successfully treated (58%). A cure rate of 58% is considered too low to justify use of this regimen in public health programs.

The new generation of quinolone antibiotics that has appeared in recent years includes ciprofloxacin, an agent with exceptionally good activity against S. typhi in vitro, with minimum inhibitory concentrations <0.06 mcg/ml. Pharmacokinetic studies in man indicate that the body fluid and tissue penetration of ciprofloxacin is excellent, including bile levels. For example, in a pilot study in which the bile levels of ciprofloxacin were measured after oral administration of 500 mg of ciprofloxacin, concentrations of drug of up to 10 mcg/ml were detected. Side effects of this antibiotic at either the 500 or 750 mg twice daily dosage schedule have been minimal. Based on these observations, we undertook a preliminary evaluation of ciprofloxacin in the treatment of chronic gall bladder carriers of S. typhi.

B. Methods and Results

Twelve chronic carriers were enrolled into the study between June and December, 1985. Patients were treated with oral ciprofloxacin 750 mg twice daily, with careful monitoring for compliance and for possible adverse effects. Therapy was stopped in two cases after 10 days: one patient had an allergic reaction and one had a minimal drop in hematocrit of uncertain etiology. The remaining patients received the complete 28 day course of drug. Stool and bile-stained duodenal string cultures were obtained before therapy and at least monthly after discontinuation of

therapy.

Of the total 12 carriers, one patient who completed the course of drug had a bacteriologic relapse within one week after completing therapy. A second patient whose stool and bile cultures were negative for six months following treatment became positive again for S. typhi. However, phage typing of the isolates showed that the organism recovered after six months of negative cultures was distinct from the original infecting strain; thus this patient represents a re-infection. The other 10 patients have remained bacteriologically negative for at least six months, including the two individuals who had their courses of therapy interrupted before the full 28 days.

These preliminary results are extremely encouraging and suggest that ciprofloxacin is efficacious in treating chronic typhoid carriers and may achieve a higher cure rate than previous antibiotic regimens. Further, more comprehensive studies will undertaken to explore this possibility.

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TABLE 1

RESULTS OF CONTROLLED FIELD TRIALS OF LYOPHILIZED ACETONE-INACTIVATED AND HEAT-PHENOL-INACTIVATED REFERENCE VACCINES

Field Site, Dates	Age Groups	Vaccine (No Doses)	No. Vaccinated	Duration of Surveillance	Incidence of typhoid per 10^5	Vaccine Efficacy	Reference
Yugoslavia, 1960-63	2-50 yrs. Mostly Schoolchildren	K (2)	5028	2 1/2 yrs.	318[a]	79%	4
		L (2)	5068	2 1/2 yrs.	727[b]	51%	
		Control (2)	5039	2 1/2 yrs.	1488[c]	—	
Guyana, 1960-67	5-15 yrs. (Schoolchildren)	K (2)	24,046	7 yrs.	71[d]	88%	5
		L (2)	24,241	7 yrs.	198[e]	67%	
		Control (2)	27,756	7 yrs.	605[f]	—	
Poland, 1961-64	5-14 yrs. (Schoolchildren)	K (2)	81,534	3 yrs.	7[g]	88%	6
		Control (2)	83,734	3 yrs.	47[h]	—	
USSR, 1962-65	Schoolchildren and young adults (92% age 7-15 yrs.)	L (2)	36,112	2 1/2 yrs.	55[i]	66%	7
		Control (2)	36,999	2 1/2 yrs.	162	—	

a vs c, $p = 0.00001$ a vs c, $p = 0.0064$ e vs f, $p < 0.000005$ g vs h, $p = 0.0000025$
b vs c, $p < 0.0004$ d vs f, $p < 0.000001$ d vs e, $p = 0.000046$ i vs j, $p = 0.000021$

TABLE 2

THE FREQUENCY OF FEVER, MALAISE AND PAIN AT THE INJECTION SITE APPROXIMATELY 24 HOURS FOLLOWING SUBCUTANEOUS INOCULATION WITH HEAT-PHENOL-INACTIVATED (VACCINE L) OR ACETONE-INACTIVATED (VACCINE K) WHOLE CELL TYPHOID VACCINES OR TETANUS TOXOID

Vaccine	No. of Vaccinees			Fever after Vaccination (%)			Inability to Work (%)	Local Pain (%)	
Group	Yugoslavia	Guyana	USSR	Yugoslavia*	Guyana+	USSR**	Yugoslavia	Yugoslavia	Guyana
Heat-phenol-inactivated	343	86	1656	24	29	6.7	23	35	54
Acetone-inactivated	326	80	-	22	26	-	21	32	45
Tetanus toxoid	328	86	1757	3	7	2.4	5	4	

* > 37°C
\+ > 37.8°C
** > 37.5°C

Data summarized from references 4, 7, and 16

TABLE 3

FIELD TRIAL OF EFFICACY OF THREE DOSES OF A LIQUID FORMULATION OF TY21A VACCINE GIVEN WITH $NaHCO_3$ TO SIX AND SEVEN YEAR OLD SCHOOLCHILDREN IN ALEXANDRIA, EGYPT. RESULTS OF THREE YEARS OF SURVEILLANCE.

Year of observation	Confirmed cases of typhoid fever	Annual incidence per 10^5	Vaccine efficacy (%)
1978-1979			
vaccinees*	0	0	100
placebo+	7	44	
1979-1980			
vaccinees	0	0	100
placebo	8	50	
1980-1981			
vaccinees	1	6	86
placebo	7	44	
Total 1978-1981			
vaccinees	1	-	96
placebo	22	-	

Data from reference 11
* n = 16, 486
\+ n = 15, 902

TABLE 4

COMPARISON OF THE EFFICACY OF TWO DIFFERENT FORMULATIONS OF TY21A VACCINE ADMINISTERED IN TWO DIFFERENT IMMUNIZATION SCHEDULES IN AREA OCCIDENTE, SANTIAGO, CHILE. RESULTS OF 33 MONTHS OF SURVEILLANCE, 9/83 - 6/86

	Enteric-Coated Capsules		Gelatin Capsules with $NAHCO_3$		
	Long Interval* (21,598)	Short Interval+ (22,170)	Long Interval (21,541)	Short Interval (22,379)	Placebo (37,793)
Cases	33	23	43	55	86
Incidence/10^5	152.8[a]	103.7[b]	199.6[c]	245.8[d]	309.4[e]
Efficacy	50.6	66.5	35.4	20.6	—

* 3 doses, 21 days between doses
\+ 3 doses, 1-2 days between doses

a vs e, p = 0.0006 a vs c, p = 0.23
b vs e, p < 0.00001 b vs d, p = 0.00052
c vs e, p = 0.023 a + b vs c + d, p = 0.001
d vs e, p = 0.21

TABLE 5

DURATION OF THE EFFICACY CONFERRED BY THREE DOSES OF THE ENTERIC-COATED CAPSULE FORMULATION OF TY21A LIVE ORAL VACCINE GIVEN WITHIN ONE WEEK IN AREA OCCIDENTE, SANTIAGO, CHILE

	Vaccine (22,170)	Placebo (27,793)
YEAR 1 (9/83-6/84)		
Cases	7	34
Incidence/10^5	31.6	122.3
Efficacy	74.2	-
YEAR 2 (7/84-6/85)		
Cases	8	23
Incidence/10^5	36.1	82.8
Efficacy	56.4	-
YEAR 3 (7/85-6/86)		
Cases	8	29
Incidence/10^5	36.1	104.3
Efficacy	65.4	-
TOTAL YEARS 1-3 9/83-6/86		
Cases	23	86
Incidence	103.7[a]	309.4[b]
Efficacy	66.5	-

* 3 doses, 21 days between doses
\+ 3 doses, 1-2 days between doses

a vs b, $p < 0.00001$

Data from references 130,133

TABLE 6

COMPARISON OF THE EFFICACY OF ONE VERSUS TWO DOSES OF TY21A LIVE ORAL TYPHOID VACCINE GIVEN IN ENTERIC-COATED CAPSULE FORMULATION. RANDOMIZED, CONTROLLED, DOUBLE-BLIND TRIAL IN AREA NORTE, SANTIAGO, CHILE

	One Dose	Two Doses	Placebo
Year 1 (7/82-6/83)	(32,788)	(27,620)	(31,948)
Cases	58	30	67
Incidence/10^5	176.9[a]	108.6[b]	209.7[c]
Efficacy	15.6%	48.2%	-
Year 2 (7/83-6/84)			
Cases	28	11	45
Incidence/10^5	85.4	39.8	140.8
Efficacy	39.3%	71.7%	-
Year 3 (7/84-6/85)			
Cases	23	15	22
Incidence/10^5	70.1	54.3	68.9
Efficacy	0%	21.2%	-
Year 4 (7/85-6/86)			
Cases	33	22	25
Incidence/10^5	100.6	79.6	78.3
Efficacy	0%	0%	-

a vs c, p = 0.42
a vs b, p = 0.037
b vs c, p = 0.0032

TABLE 7

COMPARISON OF THE EFFICACY OF TWO, THREE AND FOUR DOSES OF TY21A VACCINE IN ENTERIC-COATED FORMULATION. RESULTS OF A RANDOMIZED FIELD TRIAL IN AREA SUR AND AREA CENTRAL, SANTIAGO, CHILE

Surveillance from 10/84 to 6/26/86	Two Doses	Three Doses	Four Doses
No. of Vaccinees	94,387	95,543	58,614
Cases	121	111	32
Incidence/10^5	128.2[a]	116.2[b]	54.6[c]

a vs c, $p < 0.0001$
b vs c, $p < 0.0002$
a vs b, $p = 0.49$

Data from references 130, 133

TABLE 8

RATES OF SEROCONVERSION OF IgG-ELISA S. TYPHI O ANTIBODY FOLLOWING ONE TO THREE ORAL DOSES OF TY21A LIVE ORAL TYPHOID VACCINE GIVEN WITHIN ONE WEEK. COMPARISON OF TWO DIFFERENT FORMULATIONS.

Formulation	No. Doses	Seroconversion Rate (%)	Vaccine Efficacy in Controlled Field Trials*
Enteric-coated capsules	3	61/96 (64)	67%
	2	22/50 (44)	47%
	1	9/50 (18)	18%
Vaccine + $NaHCO_3$ in gelatin capsules	3	99/195 (50)	21%

* Data from first 36 months of surveillance in field trials in Area Norte and Area Occidente, Santiago, Chile (references 130 and 133).

TABLE 9

OCCURRENCE OF ADVERSE REACTIONS IN INFANTS AND PRE-SCHOOL CHILDREN FOLLOWING INGESTION OF LIVE ORAL TYPHOID VACCINE TY21A VACCINE OR PLACEBO

Study	Age Group	No. Doses	Diarrhea		Fever		Vomiting		Abdominal Pain	
			Vaccine	Placebo	Vaccine	Placebo	Vaccine	Placebo	Vaccine	Placebo
TyCh 6001	6-24 mos.	3	2/18*	1/19	2/18	2/19	3/18	2/19	-	-
TyCh 6003	2-5 yrs.	3	0/24	0/25	0/24	0/25	1/24	0/25	2/24	0/25
TyCh 6004	2-5 yrs.	4	1/17	2/24	1/17	0/24	0/17	1/24	1/17	2/24
Totals			3/59 (5.1%)	3/68 (4.4%)	3/59 (5.1%)	2/68 (2.9%)	4/59 (6.8%)	3/68 (4.4%)		

* No. positive/No. vaccinated

TABLE 10

SEROLOGIC RESPONSE FOLLOWING VACCINATION OF INFANTS AND YOUNG CHILDREN WITH LIVE ORAL VACCINE TY21A OR PLACEBO

Study	Age Group	No. Doses	O Antibody by IgG-ELISA		H Antibody by Widal		Vi Antibody by PHA $		Rises by Any Assay	
			V*	P*	V	P	V	P	V	P
TyCh 6001	6-24 mos.	3	0/18+	0/19	0/18	0/19	0/18	0/19	0/18	0/19
TyCh 6003	3-5 yrs.	3	5/24	0/25	3/24	0/25	0/24	0/25	8/24	0/25
TyCh 6004	3-5 yrs.	4	0/17	1/24	0/17	1/24	NT	NT	0/17	1/24
Totals			5/59	1/68	3/59	1/68	0/42	0/44	8/59	1/68

* V = vaccine group, P = placebo group
\+ No. positive/No. vaccinated
$ Positive hemagglutination using highly purified Vi antigen

TABLE 11

PREVALENCE OF IgG SPECIFIC Vi ANTIBODY
IN VARIOUS POPULATIONS MEASURED BY
ELISA WITH TYRAMINATED Vi ANTIGEN

Group & Description	GMT*	Reciprocal ELISA titer			Reciprocal PHA titer		
(No. subjects)		<50	50-100	>200	<40	80	>160
A (22) Healthy U.S. adults	26	21#	1	0	21+	1	0
B (17) Chilean Adult Acute Typhoid patients	44	15	0	2 (12%)	14	1	2
C (51) Chilean Chronic Typhoid carriers	468	6	1	44	7	5	39
D (141) Food handlers	NA	140	0	1$	139	0	2

* Geometric mean titer
\# Number of subjects with a give Vi titer
\+ Number of subjects with a given PHA titer
$ This patient was shown to be a chronic excretor of S typhi
NA Not applicable

TABLE 12

PREVALENCE OF IgM SPECIFIC Vi ANTIBODY
IN VARIOUS POPULATIONS MEASURED
BY ELISA WITH TYRAMINATED Vi ANTIGEN

Group & Description (no.)	GMT*	Reciprocal ELISA titer <100	Reciprocal ELISA titer >100
A (22) Healty U.S. adults	43	21#	1
B (17) Chilean Adult acute Typhoid patients	40	14	3
C (51) Chilean chronic Typhoid carriers	64	32	19

* Geometric mean titer
\# No. subjects with a given titer

TABLE 13

PREVALENCE OF IgA SPECIFIC Vi ANTIBODY
IN VARIOUS POPULATIONS MEASURED
BY ELISA WITH TYRAMINATED Vi ANTIGEN

Group & Description (no.)	GMT*	Reciprocal ELISA titer <50	>50
A (22) Healty U.S. adults	18	20#	2
B (17) Chilean Adult acute Typhoid patients	51	6	11
C (51) Chilean chronic Typhoid carriers	52	14	37

* Geometric mean titer
\# No. subjects with a given titer

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